

**TITLE:** Preliminary Report on The Production of a Shiga-like Toxoid Fusion Protein as Associated with Novel FliC Vaccine

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**INTRODUCTION:**

Shiga toxin (Stx) is an endotoxin produced by the bacteria *Shigella dysenteriae* while Shiga-like toxin (Stx2) is a verotoxin produced by certain strains of *Escherichia coli*. *Stx2* and *stx2B* encodes the two subunits of the holotoxin that work cooperatively to induce apoptosis in human endothelial cells. Stx2 is associated with hemolytic uremic syndrome (HUS), which causes significant morbidity and mortality in infected humans. Currently, there are no vaccines to protect against Stx or HUS.

**PURPOSE:**

Stx2B is responsible for eliciting an immune response in humans, but by itself is not toxic. The goal of the project is to introduce *stx2B* into a novel FliC cloning platform (R2-HTI)\*. This will allow the immunogenic protein encoded by *stx2b* to be expressed as a fusion protein with FliC resulting in an enhanced immune response.

**APPROACH AND RESULTS:**

*Stx2B* was subjected to multi-step PCR in order to add restriction enzyme sites compatible with the R2-HTI synthesis platform. These steps were confirmed by gel electrophoresis. The confirmed *stx2B* was ligated into R2-HTI. The ligation product, *LNDI*, was then transformed into *E. coli* DE3 cells via electroporation. These steps were then confirmed by PCR/Plasmid extraction and gel electrophoresis.

**CONCLUSION:**

Upon confirmation of *stx2B*'s incorporation into R2-HTI, *LNDI* was transformed into *E. coli* DE3 cells. Currently, induction with IPTG is being carried out in order to express the Stx2B:FliC fusion protein. Confirmation of expressing will be confirmed by SDS-PAGE/Western Blot.

**KEY WORDS:**

**Shiga-like toxin (Stx2), *stx2B*, *Escherichia coli*, Hemolytic uremic syndrome (HUS), FliC, R2-HTI, *LNDI*, PCR, gel electrophoresis, fusion protein.**

\*R2-HTI was designed by Dr. Sarah Boyd